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METHOD FOR AUGMENTING CLEANING EFFECT

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### Abstract

#### Objective

The objective of this invention is to provide a method for augmenting the cleaning effect in a system in which a bleaching agent exists. Also, this invention provides a bleaching method without increasing the density of blood stains.

#### Means to solve

A method for augmenting the cleaning effect and a bleaching method characterized by the fact that an enzyme with catalase activity is also present in a solution containing a bleaching agent. A type of bleaching agent composition or detergent composition characterized by the fact that it contains a bleaching agent and an enzyme having catalase activity.

### Claims

1. A method for augmenting the cleaning effect, characterized by the fact that an enzyme having catalase activity coexists in a solution containing a bleaching agent.

2. The method for augmenting the cleaning effect described in Claim 1, characterized by the fact that a detergent is contained in the solution containing the bleaching agent.

3. The method for augmenting the cleaning effect described in Claim 1 or 2, characterized by the fact that the enzyme having catalase activity is an enzyme that at least has a functioning pH in the range of pH 10-11.

4. The method for augmenting the cleaning effect described in any one of Claims 1-3, characterized by the fact that the enzyme having catalase activity is an enzyme for which the catalase activity at 5°C is 85% of the catalase activity at 30°C or higher.

5. The method for augmenting the cleaning effect described in any one of Claims 1-4, characterized by the fact that the enzyme having catalase activity is catalase.

6. The method for augmenting the cleaning effect described in any one of Claims 1-5, characterized by the fact that the enzyme having catalase activity is derived from Bacillus genus bacteria.

7. The method for augmenting the cleaning effect described in Claim 6, characterized by the fact that the *Bacillus* genus bacteria are *Bacillus subtilis*.

8. The method for augmenting the cleaning effect described in any one of Claims 1-7, characterized by the fact that the enzyme having catalase activity is catalase which can be prepared from *Bacillus subtilis* IAM1026 strain or IAM1069 strain.

9. The method for augmenting the cleaning effect described in any one of Claims 1-8, characterized by the fact that the bleaching agent refers to hydrogen peroxide, a hydrogen peroxide precursor, peracid, peracid precursor, and/or substances that form a reaction system that can generate hydrogen peroxide.

10. The method for augmenting the cleaning effect described in any one of Claims 1-9, characterized by the fact that the coexisting enzyme has catalase activity functions at temperatures in the range of 0-30°C.

11. A bleaching method free of an increase in the density of blood stains, characterized by the fact that an enzyme having catalase activity coexists in the solution containing a bleaching agent.

12. The bleaching method described in Claim 11, characterized by the fact that the solution containing a bleaching agent also contains a detergent.

13. The bleaching method described in Claim 11 or 12, characterized by the fact that the enzyme having catalase activity is an enzyme having a pH at least in the range of pH 10-11.

14. The bleaching method described in any one of Claims 11-13, characterized by the fact that the enzyme having catalase activity is an enzyme for which the catalase activity at 5°C is 85% of the catalase activity at 30°C or higher.

15. The bleaching method described in any one of Claims 11-14, characterized by the fact that the enzyme having catalase activity is catalase.

16. The bleaching method described in any one of Claims 11-15, characterized by the fact that the enzyme having catalase activity is derived from *Bacillus* genus bacteria.

17. The bleaching method described in Claim 16, characterized by the fact that the *Bacillus* genus bacteria are *Bacillus subtilis*.

18. The bleaching method described in any one of Claims 11-17, characterized by the fact that the enzyme having catalase activity is catalase which can be prepared from *Bacillus subtilis* IAM1026 strain or IAM1069 strain.

19. The bleaching method described in any one of Claims 11-18, characterized by the fact that the bleaching agent refers to hydrogen peroxide, a hydrogen peroxide precursor, peracid, peracid precursor, and/or substances that form a reaction system that can generate hydrogen peroxide.

20. The bleaching method described in any one of Claims 11-19, characterized by the fact that the coexisting enzyme having catalase activity functions at temperatures in the range of 0-30°C.

21. A type of bleaching agent composition, characterized by the fact that it contains a bleaching agent and an enzyme having catalase activity.

22. A type of detergent composition, characterized by the fact that it contains a bleaching agent, an enzyme having catalase activity, and a detergent.

23. A cleaning method, characterized by the fact that it makes use of the method for augmenting the cleaning effect described in any one of Claims 1-10.

24. A cleaning method, characterized by the fact that it makes use of the bleaching method described in any one of Claims 11-20.

#### Detailed explanation of the invention

[0001]

##### Technical field of the invention

This invention pertains to a method for augmenting the cleaning effect of a bleaching agent for use in preparing detergents, etc., in industry, a type of bleaching agent composition and detergent composition used in said method, and a cleaning operation adopting said method. More specifically, this invention pertains to a method for augmenting the cleaning effect by having an enzyme with catalase activity coexist in a solution containing a bleaching agent, and a novel enzyme using method pertaining to bleaching and cleaning.

[0002]

##### Prior art

When fibers, cloths, paper sheets, etc., are cleaned, usually, a bleaching agent is used. The bleaching agent may be used alone or as a component in a detergent composition that is commercially available. The bleaching agent is a substance that generates hydrogen peroxide in the cleaning solution before or during cleaning, and can become hydrogen peroxide itself or a hydrogen peroxide precursor. Usually, perborate, percarbonate, or hydrogen peroxide is used as the bleaching agent. However, for hydrogen peroxide, if the temperature is not at least 60°C, a sufficient cleaning effect cannot be realized. In order to realize an effective cleaning function at a lower temperature, or to realize a higher cleaning effect, activators which can generate peracids (such as imides, esters, or organic acid anhydrides) are added.

[0003]

However, even when such additives are added, a sufficient cleaning effect still cannot be realized at a low temperature. For example, when tap water is directly used in washing, for example, in Japan, the washing temperature is about 25°C in the summer, and lower than 5°C in the winter. In this temperature range, even for the bleaching agent containing said activator, an effective cleaning function cannot be realized. In addition, for blood stains, when said bleaching agent works, it is impossible to realize the intrinsic cleaning effect, that is, to make the color lighter or white; the color density becomes higher in many cases. Consequently, special caution should be taken in practical applications.

[0004]

Problems to be solved by the invention

The purpose of this invention is to augment the cleaning effect in a system in which a bleaching agent exists. Another purpose of this invention is to provide a bleaching method which does not increase the density of blood stains. Yet another purpose of this invention is to provide a type of bleaching agent composition or detergent composition which can be used to augment said cleaning effect or bleaching. In this invention, the cleaning effect has a broad meaning which also includes the bleaching effect.

[0005]

Means to solve the problems

In order to solve the aforementioned problems, the present inventors have performed extensive research. As a result of this research work, it was found amazingly that catalase, which decomposes hydrogen peroxide, can augment the cleaning effect of the bleaching agent that forms hydrogen peroxide in the bleaching solution. More specifically, when catalase is contained in the solution containing the bleaching agent, it is possible to bleach the black tea stains attached to clothes to a lighter color, that is, to a whiter state. In addition, for stains, such as blood stains, which are made darker due to oxidation caused by conventional bleaching agents, containing catalase in the bleaching solution can prevent this problem. This invention was reached on the basis of the aforementioned findings.

[0006]

That is, this invention provides the following items:

- 1) A method for augmenting the cleaning effect characterized by the fact that an enzyme having catalase activity coexists in a solution containing a bleaching agent.

2) The method for augmenting the cleaning effect described in 1), characterized by the fact that a detergent is contained in the solution containing a bleaching agent.

3) The method for augmenting the cleaning effect described in said 1) or 2), characterized by the fact that the enzyme having catalase activity is an enzyme that at least has a functioning pH in the range of pH 10-11.

4) The method for augmenting the cleaning effect described in any one of said 1)-3), characterized by the fact that the enzyme having catalase activity is an enzyme for which the catalase activity at 5°C is 85% of the catalase activity at 30°C or higher.

5) The method for augmenting the cleaning effect described in any one of said 1)-4), characterized by the fact that the enzyme having catalase activity is catalase.

6) The method for augmenting the cleaning effect described in any one of said 1)-5), characterized by the fact that the enzyme having catalase activity is derived from *Bacillus* genus bacteria.

[0007]

7) The method for augmenting the cleaning effect described in said 6), characterized by the fact that the *Bacillus* genus bacteria are *Bacillus subtilis*.

8) The method for augmenting the cleaning effect described in any one of said 1)-7), characterized by the fact that the enzyme having catalase activity is catalase which can be prepared from *Bacillus subtilis* IAM1026 strain or IAM1069 strain.

9) The method for augmenting the cleaning effect described in any one of said 1)-8), characterized by the fact that the bleaching agent refers to hydrogen peroxide, a hydrogen peroxide precursor, peracid, peracid precursor, and/or substances that form a reaction system that can generate hydrogen peroxide.

10) The method for augmenting the cleaning effect described in any one of said 1)-9), characterized by the fact that the coexisting enzyme having catalase activity functions at temperatures in the range of 0-30°C.

[0008]

11) A bleaching method free of increase in the density of blood stains, characterized by the fact that an enzyme having catalase activity coexists in the solution containing the bleaching agent.

12) The bleaching method described in said 11), characterized by the fact that the solution containing the bleaching agent also contains a detergent.

13) The bleaching method described in said 11) or 12), characterized by the fact that the enzyme having catalase activity is an enzyme having a pH in the range of at least pH 10-11.



14) The bleaching method described in any one of said 11)-13), characterized by the fact that the enzyme having catalase activity is an enzyme for which the catalase activity at 5°C is 85% of the catalase activity at 30°C or higher.

15) The bleaching method described in any one of said 11)-14), characterized by the fact that the enzyme having catalase activity is catalase.

16) The bleaching method described in any one of said 11)-15), characterized by the fact that the enzyme having catalase activity is derived from *Bacillus* genus bacteria.

17) The bleaching method described in said 16), characterized by the fact that the *Bacillus* genus bacteria are *Bacillus subtilis*.

18) The bleaching method described in any one of said 11)-17), characterized by the fact that the enzyme having catalase activity is catalase which can be prepared from *Bacillus subtilis* IAM1026 strain or IAM1069 strain.

19) The bleaching method described in any one of said 11)-18), characterized by the fact that the bleaching agent refers to hydrogen peroxide, a hydrogen peroxide precursor, peracid, peracid precursor, and/or substances that form a reaction system that can generate hydrogen peroxide.

20) The bleaching method described in any one of said 11)-19), characterized by the fact that the coexisting enzyme having catalase activity functions at temperatures in the range of 0-30°C.

[0009]

21) A type of bleaching agent composition, characterized by the fact that it contains a bleaching agent and an enzyme having catalase activity.

22) A type of detergent composition, characterized by the fact that it contains a bleaching agent, an enzyme having catalase activity, and a detergent.

23) A cleaning method, characterized by the fact that it makes use of the method for augmenting the cleaning effect described in any one of said 1)-10).

24) A cleaning method, characterized by the fact that it makes use of the bleaching method described in any one of said 11)-20).

[0010]

Enzyme having catalase activity

According to this invention, the enzyme having catalase activity is an enzyme that at least acts as a catalyst in the reaction of decomposition of hydrogen peroxide to oxygen and water.



[0011]

Enzymes having catalase activity are usually distributed widely in cells of aerobic organisms. Research has been performed for a long time on properties of enzymes having catalase activity. There is no special limitation on the type of the enzyme used in this invention. However, it is preferred that the pH of the cleaning solution containing bleaching agent be within the pH range for function of the enzyme. For example, because pH of the detergents commonly used in homes for washing is in the range of pH 10-11, enzymes with a functioning pH in the range of pH 10-11 are preferred. In addition, in consideration of washing at a low temperature, enzymes with little decrease in activity at a low temperature are preferred. For example, enzymes that have catalase activity at 5°C equal to 85% of the catalase activity at 30°C or higher are preferred, and enzymes that have catalase activity at 0°C equal to 95% of the catalase activity at 30°C or higher are even more preferred. In addition, in consideration of the economy of the enzymes, the enzymes that can display effects in a smaller enzyme amount are more preferred. Examples of enzymes with these properties include enzymes derived from *Bacillus* genus bacteria, or preferably enzymes derived from *Bacillus subtilis*. More specifically, enzymes with all of the aforementioned properties include those derived from *Bacillus subtilis* IAM1026 strain (registration No. FERM BP-4844) and *Bacillus subtilis* IAM1069 strain (it can be procured from Tokyo University, Molecular Cytobiology Research Lab.). Also, measurement of the catalase activity can be carried out using the method described in Japanese Kokai Patent Application No. Hei 7[1995]-246090.

[0012]

Bleaching agent

Examples of the bleaching agents include hydrogen peroxide, compounds that act as hydrogen peroxide precursors, and substances that can generate hydrogen peroxide. For example, perborate or percarbonate may be used. Also, mixtures of them with activators may be used. Examples of activators include imides, esters and organic acid anhydrides, such as TAED (tetraacetyl ethylenediamine), SNOBS (sodium nonanoyloxybenzenesulfonate), etc. However, the bleaching agent that can be used in this invention is not limited to these examples. The bleaching agents or detergents containing bleaching agents that are commercially available may be used also.

[0013]

Solution containing bleaching agent

For the solution containing bleaching agents in this invention, there is no special limitation on the method for preparing the solution, as long as it at least contains said enzyme

having catalase activity and said bleaching agent. Consequently, the solution may be prepared by adding the enzyme and bleaching agent separately or as a mixture. Also, the solution may be prepared as said enzyme and bleaching agent are added as a portion of the detergent or other component. For example, one may make use of a bleaching composition containing said enzyme alone or together with a detergent, or make use of a detergent composition containing both said enzyme and bleaching agent, or make use of a detergent composition and bleaching agent containing said enzyme.

[0014]

The concentration of said enzyme when used depends on the expected cleaning effect, type (origin) of the enzyme, the bleaching agent composition, detergent composition, cleaning conditions, etc. There is no special limitation on the concentration, which is nearly in the range of 0.001-10,000 U/mL. There is no special limitation on the concentration of the bleaching agent when used. One may make use of the commercially available bleaching agent or detergent containing the bleaching agent. The concentration may be selected to be sufficient for displaying the effect well for the specific application. Also, when the desired cleaning effect can be realized even without using the method of this patent, if the method of this invention is used anyway, it is possible to reduce the amount of the bleaching agent or detergent for realizing the same cleaning effect.

[0015]

Bleaching agent composition, detergent composition

According to this invention, one may add the enzyme having catalase activity into any conventional bleaching agent composition, detergent composition containing bleaching agent, or detergent composition used together with a bleaching agent, without changing their compositions specifically. There is no special limitation on the components of these compositions. By adding the enzyme having catalase activity to the bleaching composition, detergent composition containing bleaching agent, or detergent composition used together with a bleaching agent, one can obtain the bleaching composition, detergent composition containing bleaching agent, and detergent composition of this invention, respectively. The amount of the enzyme added should be appropriate to have the aforementioned concentration.

[0016]

For the composition of this invention, the catalase activity and bleaching agent at least in the application state are necessary requirements. As needed, the detergent component, protease, cellulase, lipase, and other enzymes may be added. Any method may be adopted to add the

enzyme into the composition of this invention. However, when the composition is in fine powder form, dust in the handling of the composition may cause problems in the safety and health of the users and the operators in the detergent industry. Consequently, this form is undesirable. On the other hand, when it is used as a solution, dust is suppressed, and the desired shape can be formed. This is preferred. Shape formation may be performed using any of the conventional methods, such as rolling pelletization, extrusion pelletization, flowing pelletization, centrifugal flowing pelletization, etc. There is no special limitation on the method for the shape of the enzyme added in the composition of this invention, as long as the shape can be formed for the composition using said methods.

[0017]

#### Application examples

In the following, this invention will be explained in detail with reference to application examples and comparative examples. Catalase derived from *Bacillus subtilis* IAM1026 strain, *Bacillus subtilis* IAM1069 strain, and *Bacillus subtilis* IAM168 strain may be prepared using the method described in Japanese Kokai Patent Application No. Hei 7[1995]-246090, followed by filtration using an ultrafiltering membrane to obtain a concentrated solution. Other types of catalase can be procured as agents on the market. Water used in washing in the various application examples was prepared by adding 73 ppm calcium chloride dihydrate in distilled water. The following compounds used in the application examples are commercial names: New Compact Attack, Liquid Wide Haiter [transliteration], Wide Haiter [transliteration] (products of Kao Corp.), Concentrated Enzyme Top, Dash, Temanashi Bright, Color Bright (products of Lion Co., Ltd.), Tide with Bleach, Ultra Ariel (products of P&G Co.).

[0018]

#### Application Example 1

Cleaning tests were performed as follows using a commercially available detergent containing a bleaching agent. Tide with Bleach, a commercially available detergent containing a bleaching agent, was dissolved in water to a standard application concentration (1440 ppm). Catalase was dissolved in the cleaning solution at a prescribed concentration. The contaminated cloth was prepared as 12 cm x 12 cm black tea-contaminated cloth BC-1 (Center for Test materials). A Terg-O-Tometer was used as the cleaning apparatus. Three pieces of said contaminated cloth were washed in 1 L of the cleaning solution at 25°C and 80 rpm for 20 min. After washing, the cloth pieces were rinsed twice, each time with 1 L water at 25°C for 3 min, followed by drying at room temperature. The enzyme effect was evaluated by means of the difference in  $\Delta Z$  between the case when the enzyme is added and the case when the enzyme is

not added,  $\Delta\Delta Z$  ( $= \Delta Z$  value when catalase is added -  $\Delta Z$  when catalase is not added). Here,  $\Delta Z$  refers to the difference in the Z-value of the contaminated cloth before and after washing ( $=$  Z-value after washing - Z-value before washing). Z-value is the Z-value of CIE pigment system, and it is measured using a color difference meter. The results are listed in Table 1.

[0019]

Table 1

//see orig. p. 5//

Key: 1 Catalase  
2 Origin  
3 Amount added  
4 Strain  
5 Cow liver  
6 Not added

[0020]

Compared with the case when catalase is not used, washing using the method of this invention displays a higher cleaning effect. Effects of this invention can be obtained irrespective of the type (origin) of the catalase. It is preferred that it originates from bacteria, or more preferably from *Bacillus* genus bacteria. These types can display significant effects at a low enzyme activity.

[0021]

#### Application Example 2

Cleaning evaluation was performed in the same way as in Application Example 1 using a combination of the commercially available bleaching agents and the commercially available detergents. Evaluation of cleaning was performed in the same way as in Application Example 1. Concentration of the bleaching agent and concentration of the detergent were those adopted in the conventional cleaning operation. For the catalase, the freeze-dried powder of the concentrated enzyme liquid derived from *Bacillus subtilis* IAM1026 strain was pelletized using

the method described in International Laid-Open Patent Application No. WO 87/04184. The pellets were then added in the detergent. The amount added was selected appropriately to have a concentration of 1 U/mL in use. The results are listed in Table 2.

[0022]

Table 2

//see orig. p. 5//

Key:	1	Detergent
	2	Bleaching agent
	3	New Compact Attack [transliteration]
	4	Concentrated Enzyme Top [transliteration]
	5	Tide with Bleach
	6	Ultra Ariel
	7	Dash
	8	Liquid Wide Powder
	9	Temamashi Bright
	10	(Contained in detergent)

[0023]

As can be seen from the results of this application example, effects of this invention can be realized independently of the types of bleaching agent, detergent, and detergent containing bleaching agent. The conventional types of bleaching agent, detergent, and detergent containing bleaching agent may be used.

[0024]

### Application Example 3

A commercially available bleaching agent was used to perform the following bleaching test. The same type of catalase as that used in Application Example 2 was used in this application example. The amount of the commercially available bleaching agent was selected so that the concentration in use was 1 U/mL. 5 g of the bleaching agent were dissolved in 1 L water, and three pieces of contaminated cloth BC-1 (12 cm x 12 cm) were loaded, followed by allowing to stand for at 25°C 1 h. Then, the cloth samples were rinsed twice, each time with 1 L water at

25°C for 3 min, followed by drying at room temperature. Evaluation of the results was performed in the same way as in Application Example 1, with results listed in Table 3.

[0025]

Table 3

//see orig. p. 5//

Key: 1 Bleaching agent  
2 Wide Haiter  
3 Color Bright

[0026]

As can be seen from the results of this application example, effects of this invention can be realized even when catalase is added to commercially available bleaching agents.

[0027]

#### Application Example 4

The test was performed in the same way as in Application Example 2, except that the standard contaminated cloth with blood stains was EMPA-116, detergent was New Compact Attack, and bleaching agent was Liquid Wide Haiter. The result was compared with the result obtained when detergent alone was used without adding of bleaching agent and catalase ( $\Delta Z = 0$ ), and the difference in the Z-value ( $\Delta Z$ ) was used as reference. The results are listed in Table 4.

[0028]

Table 4

- Key: 1      Washing system  
      2      Detergent alone  
      3      Detergent + bleaching agent  
      4      Detergent + bleaching agent + catalase

[0029]

As can be seen from the results of this application example, by containing catalase, it is possible to prevent an increase in the density of blood stains due to the bleaching agent (negative  $\Delta Z$  value).

[0030]

Application Example 5

The test was performed in the same way as in Application Example 1, except that the washing temperature was set at 5°C. The results are listed in Table 5.

[0031]

Table 5

- Key: 1      Catalase  
      2      Origin  
      3      Amount added  
      4      Strain  
      5      Not added



[0032]

As can be seen from the results of this application example, effects of this invention can be realized even at a low temperatures. However, it is preferred that enzymes with less of a decrease in activity at low temperatures, such as enzymes derived from *Bacillus subtilis* IAM1026 strain or *Bacillus subtilis* IAM1069 strain, be used.

[0033]

Effect of the invention

By adopting the method for augmenting the cleaning effect in this invention, it is possible to perform washing at a high efficiency. By using the bleaching method of this invention, it is possible to perform bleaching without an increase in the density of blood stains. Also, the bleaching composition and detergent composition of this invention can be used effectively when the aforementioned method is adopted.